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B1C
(71) Applicant
Coulter Electronics Inc
590 West 20th Street
Hialeah
Florida 33010
United States of America
(72) Inventors
David J Zahniser
Marshall D Graham
(74) Agents
Abel and Imray
Northumberland House
303–306 High Holborn
London WC1V 7LH

(54) **Disaggregation devices for cell suspensions**

(57) A disaggregating device for separating clusters of biological cells in a sample suspension, comprises: a beaker (14) containing the suspension, a rotor (12) rotatably mounted inside the beaker, a motor (18), for rotating the rotor, the rotor being spaced apart from the walls of the beaker and rotated at a sufficient speed to create shear forces on the clusters of cells located in the sample suspension between the rotor and the beaker to break up the aggregates or clusters of cells.

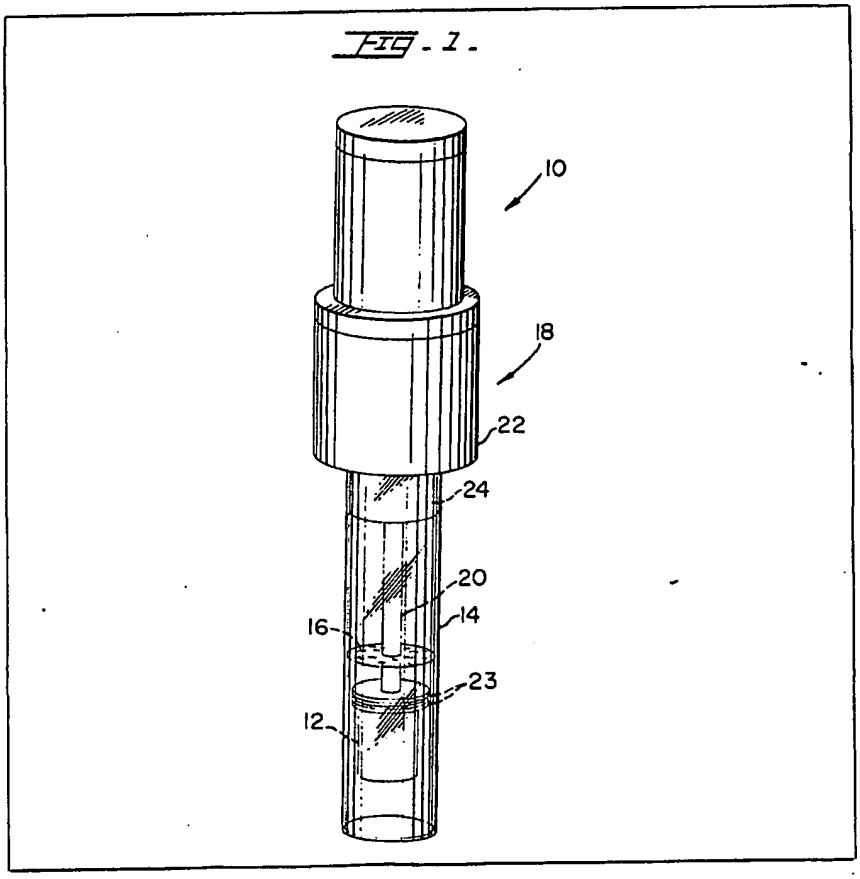

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FIG. 1.

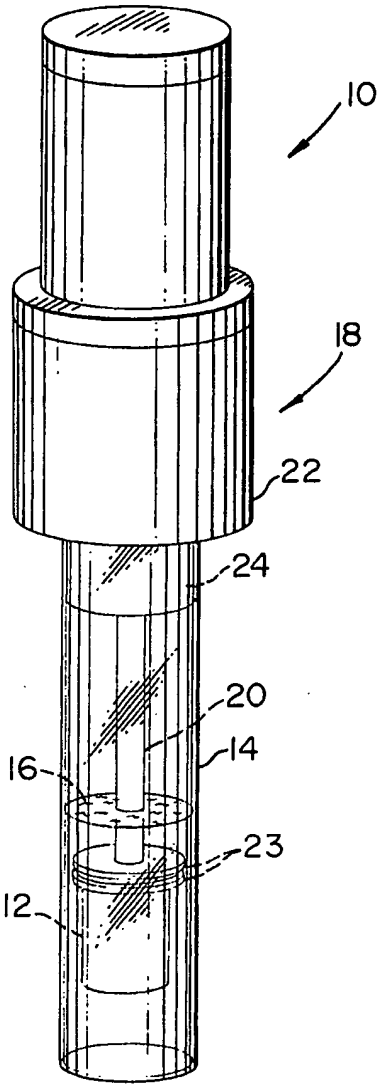
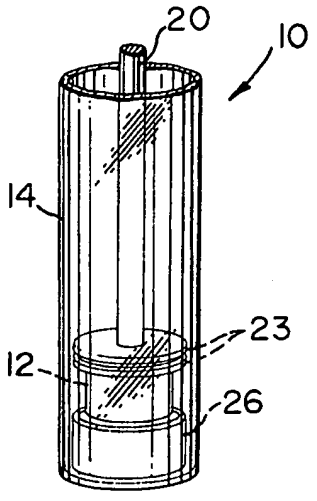


FIG. 2.



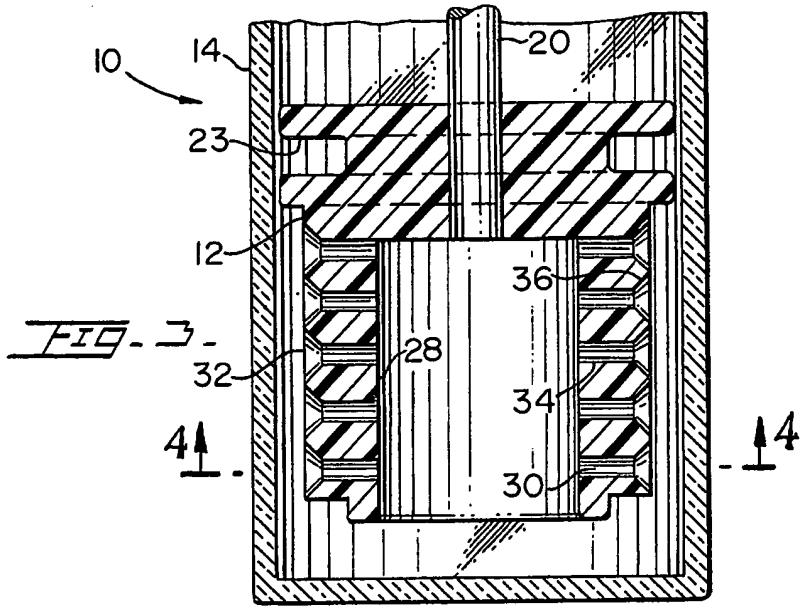


Fig. 3.

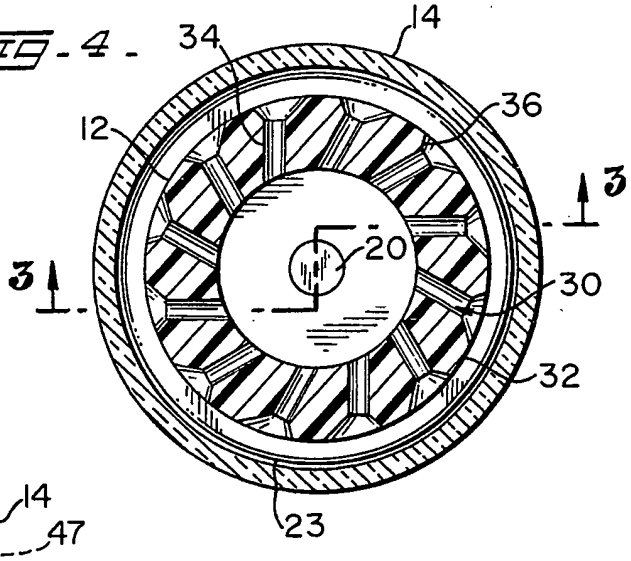


Fig. 4.

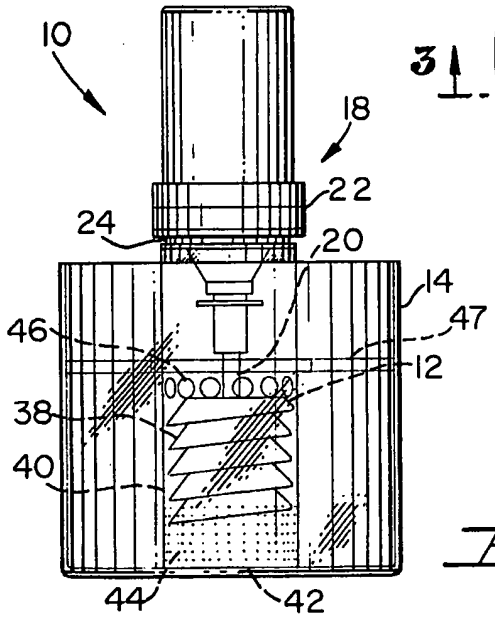


Fig. 5.

SPECIFICATION

Disaggregation devices for cell suspensions

The present invention relates to disaggregation devices for cellular suspensions to prepare samples for subsequent analysis.

Disaggregation of clusters of cells in tissue samples, scrapes, and body fluids is often desirable for visual analysis and is of extreme importance for quantitative analysis, especially where automated devices are used. Different techniques, both mechanical and chemical, have been tried to disaggregate cell clusters. For certain applications, particularly in cell cultures, the chemical techniques work well. For many applications, however, these techniques are too aggressive. Mechanical methods which have been used include shaking and stirring, forced filtration, homogenization, syringing, and ultrasonic agitation, as shown by the following articles: 1) Garcia, G.L., and Tolles, W.E., "Ultrasonic disaggregation of cell clusters", *J. Histochem. Cytochem.*, 25: 508, 1977; 2) Mayall, B.H., *"Monodisperse cell samples: the problem and possible solutions"*, in The Automation of Uterine Cancer Cytology, ed. by G.L. Wied, G.F. Bahr, and P.H. Bartels, Tutorials of Cytology, Chicago, 1976, p. 61; 3) Miller, F.,"Cytopreparatory methods: collection smearing, staining, screening, reporting," *Compendium of Cytopreparatory Techniques*, ed. by C.M. Keebler, J.W. Reagan, and G.L. Wied, Tutorials of Cytology, Chicago, 1976, p. 59; 4) Wheeless, L.L., Jr., and Onderdonk, M. A., "Preparation of clinical gynecologic specimens for automated analysis: an overview", *J. Hostochem. Cytochem.*, 22:522, 1974; and 5) Rosenthal, D.L., Stern, E., McLatchie, C., Wu, A., Lagasse, L.D., Wall, R., and Castleman, K.R., "A Simple Method of Producing a Monolayer of Cervical Cells for Digital Image Processing", *Anal. Quant. Cytol.*, 1:84, 1979. Of all the possible methods described in these articles, syringing is without a doubt the most successful method of cell disaggregation discovered to date. With syringing the cell suspension is repeatedly forced through a syringe needle. The shear forces at the tip of the needle are strong enough to break apart clusters of cells.

The present invention is directed toward a dissaggregation device for separating clusters of biological cells in a sample suspension, the device comprising a beaker containing the sample suspension, a rotor rotatably mounted inside the beaker, and means for rotating said rotor at a sufficient speed to create shear forces on the clusters of cells located in the sample suspension between the rotor and the beaker. In one modification to the device, a concentric inner vial can be positioned at the bottom of the beaker between the rotor and the inner wall of the beaker to permit collection of fractions of differing densities. In another modification of the device, an inner cavity can be formed in the rotor with a plurality of holes extending through the rotor so as to allow the centrifugal force to push liquid from the cavity through the holes. In yet another modification to the device, the rotor can be provided with a helical, worm-like outer surface and is positioned inside a cap having a plurality of large diameter holes positioned in an upper region and a plurality of small diameter holes positioned in a lower region.

By means of a device embodying the present invention, high yeilds of single cells are obtainable, with no substantial additional cullular damage due to the technique, in time periods much shorter than those previously obtainable by prior art techniques.

By way of example, only illustrative embodiments of the invention now will be described with reference to the accompanying drawings, in which:

Figure 1 is a perspective view of a first embodiment of a disaggregation device embodying the present invention;

Figure 2 is a fragmented, perspective view of a first modification of the disaggregation device shown in Fig. 1;

Figure 3 is a cross-sectional view of a second modification to the embodiment of Fig. 1 wherein the cross-section is taken with respect to sectional lines 3–3 in Fig. 4;

Figure 4 is a cross-sectional, top view of the modification shown in Fig. 3 taken along section lines 4–4; and

Figure 5 is a side view of yet another modification of the disaggregation device of Fig. 1.

A disaggregation device, generally shown by reference numeral 10, is illustrated in Fig. 1 and is operable for disaggregating or separating clusters of cells in tissue samples, scrapes, and body fluids. The disaggregated cells can subsequently be used for numerous common purposes, such as layering the cells on microscope slides for subsequent visual analysis with microscopes or by analysis with automated pattern recognition systems and for flow-through systems.

The disaggregation device 10 comprises a drum-like rotor 12 that is operable for spinning inside a round beaker 14 or like container. The rotor 12 and breaker 14 preferably have cylindrical ahapes, but can have other shapes, such as cone configurations. The beaker 14 is adapted for containing the cell sample in a liquid suspension 16. The rotor 12 is rigidly coupled to an electric motor 18 by way of shaft 20. The electric motor in turn is electrically coupled to a power source (not shown) for energizing the electric motor to turn the rotor 12. The electric motor 18 has a housing 22 with a neck portion 24 extending downward from the lower extremities of the

housing. The neck position is adapted to slidingly fit within the top of the beaker 14, so that the housing 22 will rest upon and be supported by the beaker 14. Preferably but not necessarily, one or more ledges 23 are positioned on the top portion of the rotor 12 to assist in preventing the liquid suspension 16 from moving up the inner wall of the breaker 14.

Preferably, but not necessarily, the beaker 14 is formed of plastics material, the rotor 12 is formed of plastics material, the shaft 20 is formed of metal, and the housing 22 and neck portion 24 are of plastics material. As one illustrated set of dimensions, the surface of the rotor 12 is spaced apart from the interior wall of the beaker by approximately 4 millimeters. The diameter of the rotor 12 can be, for example, 17 millimeters and the interior diameter of the beaker can be 25 millimeters. The rotor 12 is spaced apart from the floor of the beaker 14 by 1 millimeter and the vertical height of the rotor 12 is 14. 5 millimeters to the ledges 23, with the two ledges 23 adding another 5 millimeters of height. The diameter of the ledges 23 are 20 millimeters. Typically, the liquid suspension 16 might be 9 milliliters, so that the liquid level extends above the rotor 12 by 3 millimeters. These values are only given as illustrative values, and such values can vary substantially. For instance, spacings in the range of one centimeter have been used between the rotor 12 and the beaker 14, resulting in the desired disaggregation of the cells. In operation, the rotor 12 spins, creating shear forces between the outer wall of the rotor 12 and the inner wall of the beaker 14. These shear forces are sufficient to break apart cell clusters without significantly damaging the cells themselves. It is believed that these shear forces are created in a region immediately adjacent the wall of the rotor 12. The rotot 12 can be rotated, for example, between 2000 to 6000 R.P.M.'s. Generally, the faster the rotation, the better the results, with rotational speeds below 200 R.P.M.s being generally unacceptable. Tests using the device show a significant decrease in the time required to disaggregate cells, as compared to the traditional "syringing" of a cell suspension. In application of the disaggregation device 10 to cervical samples, yields of 80 to 90 perecent single cells were obtained, with no cellular damage. These results were obtained in 30 seconds of rotor use. Similar results by syringing required a disaggregation period of 10 to 15 minutes.The device 10 is well-suited for use within an automated sample preparation device. The rotor 12 is easily cleaned by operating it in a bath of running water.

Although an electric motor 18 is shown in the preferred embodiment, other means of providing energy for rotating the rotor 12 are possible. For instance, a compact air turbine drive can be readily used in place of the small electric motor 18.

Fig. 2 illustrates an adaptation of the disaggregation device 10 to permit collection of fractions of differing densities. The beaker 14 is modified to form a double-compartmented beaker by the inclusion of a concentric inner vial 26. The inner vial 26 can extend to a position below or above the bottom of the rotor 12. Preferably, the top of the inner vial 26 is positioned slightly below the bottom of the rotor 12. By selecting the intercompartmental wall placement and height of the vial 26, it is possible to collect components of the liquid suspension having differing densities, due to the centrifugal action of the rotor 12. Moreover, it is contemplated that with a plurality of concentric inner vials 26 provided along the floor of the beaker 14, it will be possible to separate the sample into more than two fractions.

Figs. 3 and 4 show another modification of the embodiment of Fig. 1. The rotor 12 has a central annular cavity 28 formed therein with a plurality of holes 30 extending from the cavity 28 to an outer wall 32 of the rotor. Each of the holes 30 has an elongated cylindrical portion 34 and truncated cone-shaped, flared portion 36. Again, the wall 32 of the rotor 12 is spaced apart from the inner wall of the beaker 14 by similar distance to that shown in Fig. 1. Each of the holes 30 is aligned so its center axis is tangent to an imaginary circle which is concentric with the shaft 20, as shown in Fig. 4. The cells to be disaggregated are drawn from the cavity 28 through the holes 30 by the centrifugal force created by the rotating rotor 12. In other words, this arrangement functions as a centrifugal pump. In addition to providing the heretofore described shearing force between the outer wall 32 of the rotor 12 and the inner wall of the beaker 14, an additional shear force is provided as the cellular material jets through the holes 30. Hence, the movement of the cells through holes 30 enhances the cells disaggregation caused by the shear force between walls.

Fig. 5 shows an alternative embodiment of the disaggregation device 10 wherein the rotor 12 has a helical cutout 38 that defines a worm-like exterior configuration. The beaker 14 is provided with greater cross-sectional dimensions relative to the beaker sizes of the pevious embodiments. A closed sleeve or cap 40 is interposed between the rotor 12 and the beaker 14. More specifically, the electric motor 18 now rests upon the top of the cap 40. The cap 40 has a closed end 42 which sits in the bottom of the beaker 14. A plurality of small diameter holes 44 pass through the cap in its lower regions toward the lower end of the rotor 12. A plurality of large diameter holes 46 pass through the cap 40 in a region in the vicinity of the top of the rotor 12.

Preferably, but not necessarily, a ledge 47 is mounted to the beaker 14 at a height in the vicinity of the top of the liquid sample to prevent the liquid suspension from moving up the walls. With the direction of the helical cutout 38 shown in Fig. 5, the clockwise rotation, as seen from below, of the rotor 12 forces liquid downward toward the bottom of the cap 40. By virtue of this arrangement, liquid from the beaker 14 is drawn through the large diameter holes 46, is forced downward toward the bottom of cap 40 and proceeds outward through the small diameter holes 44. In addition to the shear force created between worm-like shaped surface of the rotor 12 and the inner wall of the beaker 14, additional shear forces are created as the liquid squirts out of the small diameter holes 44. Moreover, compared to the arrangement of Figs. 3 and 4, the rotation of the helical cutout 38 allows for the development of substantially greater pressures for forcing the cells through the holes 44. More specifically, the combination of the shear force of the rotor 12 and the holes 44 in the cap 40 provides excellent disaggregation with up to 10 percent improvement over the basic rotor device shown in Fig. 1. This added pressure also can be useful for stripping the cytoplasm from cell nuclei.

Although particular embodiments of the invention have been shown and described herein, the intention covers all modification, alternatives, embodiments, usages and equivalents of the subject invention as fall within the scope of the invention as defined in the appended claims.

CLAIMS

1. A disaggregation device for separating clusters of biological cells in a sample suspension, comprising: a beaker containing said sample suspension; a rotor rotatably mounted inside said beaker; and rotating means for rotating said rotor to create shear forces to disaggregate said clusters of cells located in the sample suspension between said rotor and said beaker.

2. A disaggregation device according to claim 1, wherein the outer surface of said rotor and the inner wall of said beaker have annular cross-sectional configurations.

3. A disaggregation device according to claim 1 or 2, wherein said rotor has a lower end which is positioned in spaced-apart relationship to the bottom of said beaker.

4. A disaggregation device according to any one of claims 1, 2 or 3, further including, an inner vial positioned between said rotor and said beaker at the bottom of said beaker.

5. A disaggregation device according to claim 4, wherein said inner vial has a circular cross-sectional configuration and is positioned in concentric relationship with said beaker and said rotor and said inner vial has a height that extends upward proximate to the vicinity of the lower end of said rotor.

6. A disaggregation device according to any one of claims 1, 2 or 3, in which: said rotor has a cavity formed therein; a plurality of holes pass through said rotor, and said holes extend from said cavity to the outer surface of said rotor; whereby the rotation of said rotor creates a centrifugal force that moves the sample from said cavity through said holes.

7. A disaggregation device according to any one of claims 1, 2 or 3, further comprising: a cap positioned inside of said beaker; said cap having a plurality of relatively small diameter holes and a pluraltiy of relatively large diameter holes, all passing through the wall of said cap, with said large diameter holes being positioned above said small diameter holes; and said rotor having on its surface a helical cutout; whereby rotation of said rotor pulls the sample suspension through said large diameter holes and pushes the sample suspension downward inside of said cap so as to force the sample suspension through said small diamete holes.

8. A disaggregation device according to claim 7, wherein said large diameter holes are located at or above the upper portion of said rotor and said small diameter holes are located at or below the lower portion of said rotor.

9. A disaggregation device for separating clusters of biological cells in a sample suspension, the device being substantially as herein described with reference to, and as illustrated by, Fig. 1 of the accompanying drawings.

10. A device as claimed in claim 9 but modified substantially as herein described with reference to, and as illustrated by, Fig. 2 of the accompanying drawings.

11. A device as claimed in claim 9 but modified substantially as herein described with reference to, and as illustrated by, Figs. 3 and 4 of the accompanying drawings.

12. A device as claimed in claim 9 but modified substantially as herein described with reference to, and as illustrated by, Fig. 5 of the accompanying drawings.

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